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Chapter 6: Evaluation of Storage and Analysis Protocols for Environmental Water Samples Containing Ethanol

Author

Carolyn Koester*

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***Lawrence Livermore National Laboratory, Livermore, CA 94550**

University of California • Livermore, CA 94550

Lawrence Livermore National Laboratory

Environmental Restoration Division

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Summary

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Improved routine sampling and analysis methods are needed to meet the data quality objectives of future studies and groundwater resource management. A study was performed to evaluate the best storage and contract laboratory analytical protocols for environmental water samples containing ethanol and to make recommendations for improvement. This study found that:

- Ethanol in samples can be easily degraded and that care should be taken to preserve a sample as quickly as possible after sample collection.
- Refrigeration without preservation does little better than no preservation at all.
- Acidification of groundwater samples followed by refrigeration adequately preserves ethanol in groundwater samples for longer than two weeks.
- It is reasonable to expect that analytical laboratories should be able to achieve reporting limits of 50-500 *ppb* for ethanol in clean water. All commercial laboratories that were part of this study were capable of accurately detecting ethanol in clean groundwater at concentrations near their reporting limits.
- Reporting limits will be increased in the presence of interferences caused by other analytes, such as gasoline components. In this case, the actual reporting limits achievable will depend on analyst experience and the extent to which the sample must be diluted prior to analysis.
- To document the skill with which a contract lab handles complex samples, the individual submitting samples is encouraged to send known performance evaluation samples to the contractlaboratory.

6. Evaluation of Storage and Analysis Protocols for Environmental Water Samples Containing Ethanol

6.1. Introduction

Ethanol is a possible substitute for the gasoline oxygenate methyl tertiary butyl ether (MTBE). MTBE has been found as a contaminant in many environmental water samples and has been the source of public concern (USEPA, 1997). Ethanol, which is readily biodegraded (Malcolm Pirnie, Inc., 1998), is viewed as a good replacement for MTBE. In April 1999, gas stations around Lake Tahoe (California) began selling MTBE-free gasoline, which could contain as much as 5% ethanol (Singer, 2000). After this change, ethanol was measured in groundwater monitoring wells around this region, suggesting that ethanol was leaking from underground storage tanks (Singer, 2000).

In order to determine the extent to which ethanol, released in pure form or as part of a gasoline mixture, may impact ground and surface waters, it is critical to accurately determine ethanol concentrations in groundwater samples, both with and without the presence of other gasoline fuel hydrocarbons. An important step in making an accurate assessment is to collect a representative environmental water sample and to preserve it until an analysis can be performed. If the sample is not properly handled, the ethanol concentrations measured by the analytical laboratory will not accurately reflect environmental concentrations. In the case of ground or surface water containing ethanol, the major concern is to prevent biodegradation of the ethanol.

Little has been reported regarding the collection and preservation of environmental water samples containing ethanol. Conventional United States Environmental Protection Agency (USEPA) methods for sample collection and storage of volatile organic compounds are often applied (i.e., collection in 40-milliliter, glass vials with Teflon®-lined septum caps and storage at 4°C, with or without a preservative, for a maximum of 14 days prior to analysis) [Keith, 1996]. These storage methods have not always been rigorously tested; this is especially true for samples containing ethanol because ethanol is not currently an environmental contaminant of concern. Researchers determining ethanol in biological fluids have refrigerated (Macchia *et al*., 1995) or frozen (Macchia *et al*., 1995; Mc-Carver-May *et al*., 1997; Tangerman, 1997) samples prior to analysis. However, the matrices of biological fluids are distinctly different from environmental waters. Ethanol preservation strategies of the biology community might not apply to the analysis of ethanol in ground or surface water samples. For this reason, we chose to investigate whether the preservation strategies of refrigeration (either alone or with acidification, and with filtering) and freezing were applicable to ethanol analysis.

In addition, we wished to determine if routine contract analytical laboratory methods were sufficiently developed to provide accurate measurement of ethanol in environmental waters. Ethanol, a very water-soluble (polar) molecule, is challenging to analyze because it is difficult to extract from water. Once ethanol is extracted from an aqueous matrix, sufficient methods exist to separate, detect, and measure its concentration in solution. The most common strategies for ethanol analyses use gas chromatography (GC). These include heated headspace extraction

followed by GC with flame ionization detection (FID) (Macchia *et al*., 1995; Mc-Carver-May *et al*., 1997; Watts *et al*., 1990) and direct injection of a sample followed by GC/FID (Tangerman, 1997; Corseuil, 1998; USEPA, 1996a) or two-dimensional GC/FID (ASTM, 1997). GC/mass spectrometry (MS) with purge and trap sample introduction (USEPA, 1996b) or with solid-phase microextraction (SPME) sample introduction (NWRI, 1999) has been used to determine ethanol in environmental samples.

6.2. Materials and Methods

6.2.1. Materials

Clean groundwater was obtained from a local well. This water was pH 7.2, had a specific conductance of 900 µmhos/cm, a total alkalinity of 300 mg/L (measured as $CaCO_3^-$), a sulfate concentration of 44 mg/L, a chloride concentration of 90 mg/L, and approximately 500 mg/L total dissolved solids. This water contained no measurable amounts of ethanol (50 ppb reporting limit) or any of the volatile organic compounds listed in EPA Method 8260 (0.5 ppb reporting limit). Ethanol was obtained from Aldrich (Milwaukee, WI) and *n*-propanol was obtained from Burdick and Jackson (Muskegon, MI). Hydrochloric acid was obtained from Baker (Phillipsburg, NJ). Deionized water was obtained from a Milli-Q system, Model Gradient A-10 (Millipore, Bedford, MA) and was heated and purged for several hours prior to use. Purged water from the Milli-Q system was used for laboratory control samples and blanks.

6.2.2. In-house Ethanol Analysis Protocol

In-house ethanol analyses were performed using purge and trap GC/MS. Fresh ethanol and *n*-propanol standards, in purged water, were made daily. The instrumentation used consisted of a Dynatech Model PTA-30 autosampler, an OI Analytical (College Station, TX) 3100 purge unit, and a Hewlett Packard 5971 GC/MS system (Palo Alto, CA). A 20-mL water sample, to which 1 mg/L of isopropanol had been added, was heated to 65ºC and purged with 40 cc/min of helium for 11 minutes. The isopropanol was used to verify that the GC/MS was operating optimally during all analyses. Purged volatile organic compounds (VOCs) were transferred, via a 110°C transfer line, to an OI Analytical #6 trap. Desorption of analytes from the trap occurred as the trap was heated ballistically to 180°C for two minutes. The analytes were transferred, via another 110°C transfer line, to the 250°C injection port of the GC/MS. Separation was performed using a 60-m, RTX 502.2 column (Restek Corp., Bellefonte, PA), with 0.32-mm i.d. and 2-µm film thickness. The GC was held at 35ºC for four minutes, heated at 6ºC/min to 150ºC, heated at 15ºC/min to 220ºC, and held at 220ºC for five minutes. The MS was operated in the full-scan mode and was scanned from 29 atomic mass units (amu) to 100 amu at a rate of 1.9 scans/sec. The mass chromatogram of *m/z* 31 was used for alcohol quantitation, which was performed using external standard calibration. Method detection limit, as determined by the standard EPA definition (Glaser *et al*., 1981) and analyzing eight replicate water samples containing 0.5-mg/L ethanol was 0.05 mg/L. However, because trace concentrations of ethanol were often observed in our blank samples, a more realistic reporting limit of 0.5-mg/L ethanol was adopted.

6.2.3. Storage Study Sample Treatments

6.2.3.1. High Concentration (5 mg/L)

A known amount of neat ethanol was added to a gallon of groundwater such that its final concentration was approximately 5 mg/L. Because of the difficulties associated with mixing large volumes (>4L) of solution, final concentrations of the ethanol in these samples were determined by GC/MS. Approximately 30, individual, amber, 40-mL volatile organic analysis (VOA) vials with Teflon-lined septa were filled (leaving no headspace) with this solution and stored at ambient conditions. Another 30 vials were filled with this solution and refrigerated at 4°C. Thirty vials were acidified to pH<2 with six drops of a 50% hydrochloric acid solution and refrigerated. Thirty, 125-mL, HDPE plastic bottles were filled with approximately 40 mL of ethanol spiked groundwater and immediately frozen at -20°C. An additional 8-mg/L ethanol solution in groundwater was made, passed through a 0.2-µm nitrocellulose filter (Millipore, Bedford, MA), and stored in individual VOA vials. At varying intervals, three individual containers, representing each storage condition, were sampled and analyzed for ethanol. All analyses for the high concentration storage study were performed in-house.

6.2.3.2. Low Concentration (200 ppb)

Known amounts of neat ethanol (Chem Service, Inc., West Chester, PA) were added to two, one-liter bottles of groundwater such that the final ethanol concentration in each bottle was approximately 200 ppb. Final concentrations of the ethanol in these samples were determined by GC/MS. Approximately 30, individual, amber, 40 mL-VOA vials with Teflon-lined septa were filled (leaving no headspace) with this solution, which was acidified to pH<2 with hydrochloric acid and refrigerated at 4°C. Thirty VOA vials were filled with 200-mg/L ethanol solution that had been passed through a 0.2-µm filter, as described above. These vials were also refrigerated (unacidified) at 4°C. At varying intervals, replicate containers were sampled and analyzed for ethanol by a contract laboratory.

6.2.4. Laboratory Comparison Study

To evaluate the holding times for environmental water samples containing high concentrations of ethanol (~5 mg/L), we performed in-house analyses. To evaluate the holding times for environmental water samples containing lower concentrations of ethanol (200 ppb), we sent samples to a contract lab, with better detection limits, for analyses. To evaluate the analytical methods that may routinely be used by laboratories, we sent replicate groundwater samples containing various concentrations of ethanol to several different laboratories for ethanol analysis by purge and trap GC/MS and by direct injection GC/FID. We then compared the analytical results generated with the known concentration of ethanol in each sample.

Several samples of laboratory water or groundwater were spiked with known concentrations of ethanol (ranging from 0.05 to 50 mg/L) and/or 50 mg/L RF-A2 gasoline (American Petroleum Institute, Washington, DC). These samples were acidified to pH<2 and were analyzed by different contract laboratories. Laboratories used either EPA Method 8260 (purge and trap followed by GC/MS) (USEPA, 1996b) or EPA Method 8015 (direct injection followed by GC with FID (USEPA, 1996a). Table 6-1 summarizes the methods used by each laboratory.

6.3. Results

6.3.1. Sample Storage Studies

Ethanol (5 mg/L) in a groundwater sample that was refrigerated at 4°C degraded to a concentration below the reporting limit of 0.5 mg/L within four days (Figure 6-1). In comparison, ethanol in a groundwater sample that was stored at ambient conditions was degraded to a concentration below the reporting limit within two days. Within one day, seventy percent of the ethanol in this solution had degraded (Figure 6-2). Clearly, the strategy of sample preservation by refrigeration alone and analysis within 7–14 days of collection, as advocated in some EPA methods, is not sufficient for the preservation of ethanol in a sample. This is in contrast to data from biological samples, where it has been observed that ethanol in blood was stable for two weeks when stored at room temperature or refrigerated (Tangerman, 1997). Ethanol was stable for seven days in urine, serum, plasma, and saliva when stored at 4°C (Macchia *et al*., 1995).

EPA suggests that samples can be preserved by acidification to pH<2 and storage at 4°C. This protocol works well for ethanol preservation. Ethanol in a groundwater sample at concentration of 6 mg/L was stable for over a month (Figure 6-3). We also examined this storage protocol for its applicability to a lower concentration of ethanol. Ethanol, at approximately 200 ppb (0.2 mg/L), in a groundwater sample that was acidified and refrigerated was also found to be stable after 14 days of storage (Figure 6-4). Thus, sample acidification is an effective storage protocol.

We assumed that bacteria present in the water samples may have caused ethanol degradation; therefore, removing the bacteria with a 0.2-µm filter should reduce the bacterial population such that ethanol in a sample would not degrade. This preservation strategy is desirable to investigate because it does not require the use of hazardous chemicals. However, sample filtering might not be appropriate for sample preservation if the sample is to be analyzed for more volatile compounds, such as benzene, toluene, and xylenes—these hydrophobic and volatile components would be expected to volatilize during the filtering process.

We first determined that, because ethanol is miscible with water, its adsorption to filter media and its volatilization from a sample during the filtering process were not observed (data not shown for 5 mg/L and 200 ppb of ethanol in water). When filtering was performed prior to refrigeration, samples containing 8-mg/L ethanol were stable for two weeks (Figure 6-5). While showing no significant changes in concentration after 10 days of storage, 200-ppb ethanol disappeared from the sample after 14 days in storage (Figure 6-6). This sample may have become contaminated with bacteria from another source. Because it is difficult to completely eliminate bacteria from a sample and to prevent the introduction of bacteria into the sample from outside sources, filtering is not the best option for sample preservation.

Water, serum, and urine samples containing ethanol have been stored for many months, or even years, at -20°C without ethanol loss (Machia *et al*., 1995; Tangerman, 1997). Thus, we also tested if storage at -20°C would be sufficient for the preservation of 5-mg/L ethanol in groundwater. Figure 6-7 confirms that freezing provided adequate sample preservation for at least one month (the duration of this study). This preservation option might be useful only to research laboratories that have immediate access to -20°C freezers.

6.3.2. Contract Analytical Laboratory Comparisons

The two methods for ethanol analysis used by the contract analytical laboratories in this study were purge and trap GC/MS and direct injection GC/FID. Table 6-1 summarizes the merits of the methods used by each laboratory. Of the methods used in this study, GC/MS afforded the best detection limits. The laboratory that used GC/FID had a reporting limit for ethanol of 5 mg/L, while the laboratories that used GC/MS claimed reporting limits of 0.005–1 mg/L. In addition, GC/MS provides more specific analyte detection than GC/FID. GC/MS provides retention time data and mass spectral data for each compound eluting from the GC column. GC/FID provides only retention time data, which is why Lab #4 needed to analyze each sample on two different GC columns to confirm the identity of each analyte detected.

Laboratories performing standard methods to measure volatile organic compounds (VOCs) typically use methanol as a solvent for the surrogate and internal standards. The surrogate and internal standard solutions are added to a sample prior to analysis. This practice adds approximately 4 parts-per-thousand of methanol to the sample. This can cause a problem for ethanol determinations because methanol and ethanol often co-elute from the standard GC columns used for volatile compound analyses (i.e., the signal from the methanol in the sample obscures the ethanol's signal).

Thus, in order to determine ethanol, either methanol must be eliminated from the sample or the GC column and conditions must be selected such that methanol and ethanol do not co-elute. Lab #1 and Lab #4 chose to use water to dissolve the surrogates used in the analyses. A disadvantage to using water as a solvent for the standards is that the standard solutions need to be prepared daily. Because water was used as the solvent, these labs used water-soluble analytes as surrogates. Lab #1 used *n*-propanol as a surrogate and Lab #4 used isobutanol. Lab #2 chose to substitute *n,n*-dimethyl formamide as a solvent for their surrogate standards. This compound has a higher boiling point than ethanol and is easily resolved from ethanol under standard GC conditions. Lab #3 and Lab #5 opted to use methanol as a solvent for their standards. The use of cryo-focussing by Lab #3 assured that methanol and ethanol could be resolved on a relatively short (30 m) GC column. Lab #5 used a relatively long (105 m) column, which easily separated ethanol and methanol; in addition, this laboratory operated the MS such that ions from methanol would not be detected.

For the first round of analyses, ethanol was spiked, at known concentrations, into clean laboratory water and into clean groundwater so that the analytical laboratories were able to perform ethanol analyses under the best possible conditions (in a simple, clean matrix). We first established that no interferences or laboratory contamination were measurable in the blank samples. No ethanol was detected by any of the labs in clean groundwater or in laboratory water (Table 6-2, Samples A and B).

The best detection limits of ethanol were observed in laboratories using GC/MS. Only Lab #2 and Lab #3, who claimed the reporting limits of 0.005 mg/L and 0.05 mg/L, respectively, detected 0.072 mg/L of ethanol in clean groundwater. The concentrations of ethanol measured by these labs were within 10% of the known values (Table 6-2, Sample C). These two labs also accurately (agreement within 10% of known values) measured 0.112 mg/L of ethanol in groundwater (Table 6-2, Sample D). Note that, while Lab #1 also used GC/MS and could, by a standard definition of detection limit, measure 0.05 mg/L of ethanol, this laboratory was not able to report results below 0.5 mg/L because they often found trace levels of ethanol in blanks.

Labs #1, #2, #3, and #5 (all using GC/MS) detected ethanol in a sample containing 1.12 mg/L ethanol (Table 6-2, Sample D). Labs #3 and #5 measured values that were with 16% of the known value. Lab #2 measured a value that was 35% higher than the known value. Lab #1 measured ethanol at a concentration that was 60% higher than the known value. One possible cause might be that Lab #1 was the only laboratory to use an external standard for GC/MS analysis; an internal standard method, which accounts for fluctuations in instrument performance, should be used to provide better data.

All labs easily detected (within 25% accuracy) 11.2 mg/L ethanol in groundwater (Table 6-2, Sample F). The largest deviations from true values were Lab #2 and Lab #3. Note that Lab #2 performed a 40-fold dilution of the sample prior to analysis.

While it is easy to determine ethanol in a clean sample, detection of ethanol is expected to be more difficult in a more complex sample matrix. For this reason, a second round of analyses was performed to determine the effect of gasoline as a potential interference. In environmental samples, ethanol attributed to leaking underground storage tanks will be found with gasoline. Thus, we added approximately 50-mg/L gasoline to several samples which were to be analyzed for ethanol. To obtain this concentration of gasoline in the samples, 3 µL of neat gasoline was added directly to the 40 mL VOA vials prior to their shipment to the laboratories. This was done so that we would not be required to use a co-solvent, which could potentially interfere with the ethanol analyses.

Again we verified that the laboratories did not detect ethanol in the blank samples (Table 6-3, Sample G) and that the ethanol was not detected in groundwater that was spiked with gasoline only (Table 6-3, Sample H). Note that with 50-mg/L gasoline in the samples, Labs #2 and #3 found it necessary to dilute the samples prior to analysis. Under these conditions, the laboratories would not be able to achieve optimal reporting limits.

The data in Table 6-3, Sample I, indicates that neither Lab #3 nor Lab #5 were able to detect ethanol at a concentration that was two-fold greater than their best reporting limits. Note that both of these laboratories were able to detect ethanol at 10 times their stated reporting limits with 50-mg/L gasoline present (Table 6-3, Sample J). The accuracy of the laboratories in determining ethanol at ten-times the stated reporting limit in gasoline-spiked water was equally as good as determining ethanol in clean groundwater (Table 6-3, Samples J and K).

Note that the two laboratories using GC/MS found it necessary to dilute samples containing 50-mg/L gasoline prior to analysis. It is not realistic to expect that laboratories will meet their stated reporting limits in complex matrices. Thus, detection/reporting limits should ideally be established for each matrix to be studied. As this is not always practical, the user will need to rely on the expertise of the laboratory in processing samples. The data from Lab #2 shows that the reporting limits obtained are dependent on the analyst's experience. Table 6-3 data for Lab #2 shows that although the concentration of gasoline interference remained constant at 50 mg/L, different samples were analyzed using different dilution factors. These dilution factors were not correlated with either gasoline or ethanol concentrations in these samples.

6.4. Conclusions

Data suggest that ethanol in samples can be easily degraded and that care should be taken to preserve a sample as quickly as possible after sample collection. Our study suggests that

refrigeration without acidification does little better than no preservation at all. Acidification of groundwater samples followed by refrigeration adequately preserves ethanol in groundwater samples for longer than two weeks. Sample acidification (to pH<2) can be performed in the field by placing samples in commercially available VOA vials that contain a small (<1 mL) amount of hydrochloric acid. The exact quantity of acid needed for sample preservation will depend on the buffering qualities of the waters being sampled. Note that, if sample preservation by acidification is used, samplers should be informed of the potential danger of working with hydrochloric acid and should be trained regarding the proper use of personal protective equipment, such as safety goggles and gloves, and the proper procedure for sample transport and shipping.

All laboratories were capable of accurately detecting ethanol in clean groundwater at concentrations near their reporting limits (although, we did not evaluate if Lab #2 could meet its reported 0.005 mg/L reporting limit). It seems reasonable to expect that good laboratories should be able to achieve reporting limits of 50–500 ppb for ethanol in clean water. Reporting limits will be reduced in the presence of interferences, such as gasoline. In this case, the actual reporting limits achievable will depend on analyst experience and the extent to which the sample must be diluted prior to analysis. To document the skill with which a contract lab handles complex samples, the individual submitting samples is encouraged to send known performance evaluation samples to the contract laboratory.

While purge and trap or direct injection sample introduction were used by the contract laboratories because they were readily available and easy to perform, the methods of azeotropic distillation (USEPA, 1996c) and vacuum distillation (USEPA, 1996d) prior to GC/MS or GC/FID analysis might provide better detection limits and should be investigated.

6.5. Recommendations

- Any environmental water samples submitted for ethanol analyses should be preserved with hydrochloric acid to pH <2 and analyzed within two weeks.
- Ethanol concentrations measured in unpreserved water samples should be considered suspect. Ethanol concentrations in unpreserved samples might be artificially low because of ethanol biodegradation.
- GC/MS is the preferred method of ethanol analysis.
- Laboratories using GC/MS should be able to obtain reporting limits (in a clean sample matrix) of 50–500 parts-per-billion; however, in complex matrices (i.e., highly contaminated waters or samples that require dilution prior to analysis), even excellent laboratories will not be able to achieve the reporting limits claimed for clean matrices. In these cases, reporting limits that are ten-fold (or more) greater than reporting limits in clean matrices are to be expected.
- In evaluating the quality of data produced by contract laboratories, the individuals submitting samples should include periodic performance evaluation samples.

6.6. Acknowledgements

Ethanol analyses were performed by BC Laboratories (Bakersfield, CA), and Kiff Analytical (Davis, CA), Severn Trent Laboratories (Earth City, MO), and Zymax (San Luis Obispo, CA). Zymax also provided ethanol analyses for the low concentration storage study.

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Tables

Table 6-1. Brief description of laboratory methods used for ethanol analyses.

	Lab #1	Lab #2	Lab #3	Lab #4	Lab #5
Detector	MS	MS	MS	FID	MS
GC column	60 m × 0.32 mm i.d., DB-624	60 m × 0.32 mm i.d., RTX 502.2	30 m × 0.25 mm i.d., DB-5	30 m × 0.53 mm i.d., RTX-5 and RTX-200	105 m × 0.53 mm i.d., RTX 502.2
Sample size (mL)	20	5	5	0.001	25
Sample Introduction Method	Purge & trap	Purge & trap	Purge & trap	Direct injection	Purge & trap
Report Limit (mg/L)	0.5	0.005	0.05	5	1
Solvent for Standards	Water	*n,n*-dimethyl formamide	Methanol	Water	Methanol
Quantitation	External standard	Internal standard	Internal standard	External standard	Internal standard
Reproducibility (relative difference for duplicate ethanol analyses using lab water)	11% (data set I) for 0.5 mg/L EtOH	Not provided	24% (data set I) 10% (data set II) for 0.5 mg/L EtOH	4% (data set I) 19% (data set II) for 50 mg/L EtOH	Not provided

Notes:

DB =

EtOH =

FID = Flame ionization detector.

GC = Gas chromatography.

i.d. = Inside diameter.

m = Meters.

Mg/L = Milligrams per liter.

mL = Milliliter.

mm =

MS = Mass spectrometry.

Table 6-2. Ethanol (in mg/L) measured in clean groundwater spiked with known concentrations of ethanol.

Sample	Water type	EtOH added (mg/L)	Measured EtOH concentration (mg/L)				
			Lab #1	Lab #2	Lab #3	Lab #4	Lab #5
A	Ground	0	<0.5[a]	<0.005[a]	<0.05[a]	<5[a]	<1[a]
B	Lab	0	<0.5[a]	<0.005[a]	<0.05[a]	<5[a]	<1[a]
C	Ground	0.072	<0.5[a]	0.073 (+1%)	0.067 (-7%)	<5[a]	<1[a]
D	Ground	0.112	<0.5[a]	0.11 (-1%)	0.1 (-10%)	<5[a]	<1[a]
E	Ground	1.12	1.8 (+60%)	1.5 (+34%)	0.99 (-11%)	<5[a]	1.3 (+16%)
F	Ground	11.2	12 (+7%)	14 (+25%)[b]	14 (+25%)	12 (+7%)	11 (-2%)[c]

Note:

Laboratory water and clean groundwater served as blank control samples. "ND" indicates that ethanol was not detected at the reporting limits found in Table 1. The numbers in parentheses indicate the percent differences between known and measured ethanol concentrations.

mg/L = Milligrams per liter.

EtOH = Meters.

[a] Ethanol was not detected at the stated reporting limit.

[b] Sample was analyzed at a 40–fold dilution.

[c] Sample was analyzed at a 2–fold dilution.

Table 6-3. Ethanol (in mg/L) measured in clean groundwater or groundwater containing 50 mg/L of gasoline spiked with known concentrations of ethanol.

Sample	Matrix	EtOH in sample	Measured EtOH concentration (mg/L)			
			Lab #2	Lab #3	Lab #4	Lab #5
G	groundwater	0	<0.005[a]	<0.05[a]	<5[a]	<1[a]
H	groundwater + 50 mg/L gas	0	<0.01[a,b]	<0.2[a,c]	<5[a]	<1[a]
I	groundwater + 50 mg/L gas	2 × RL	not analyzed	<0.1[1,4]	not analyzed	<1[a]
J	groundwater + 50 mg/L gas	10 × RL	not analyzed	0.57 (+14%)[b]	not analyzed	10 (-1%)
K	groundwater	10 × RL	not analyzed	0.53 (+6%)	not analyzed	11 (+10%)
L	groundwater + 50 mg/L gas	50 × RL	not analyzed	2.7 (+8%)[d]	not analyzed	not analyzed

Note:

Clean groundwater served as blank control samples. "ND" indicates that ethanol was not detected at the reporting limits ("RL") found in Table 1. The numbers in parentheses indicate the percent differences between known and measured ethanol concentrations.

[a] **Ethanol not detected at reporting limit indicated. Reporting limits were adjusted to account for sample dilution prior to analysis.**

[b] **Sample analyzed at a 2-fold dilution.**

[c] **Sample analyzed at a 40-fold dilution.**

[d] **Sample analyzed at a 20-fold dilution.**

Figures

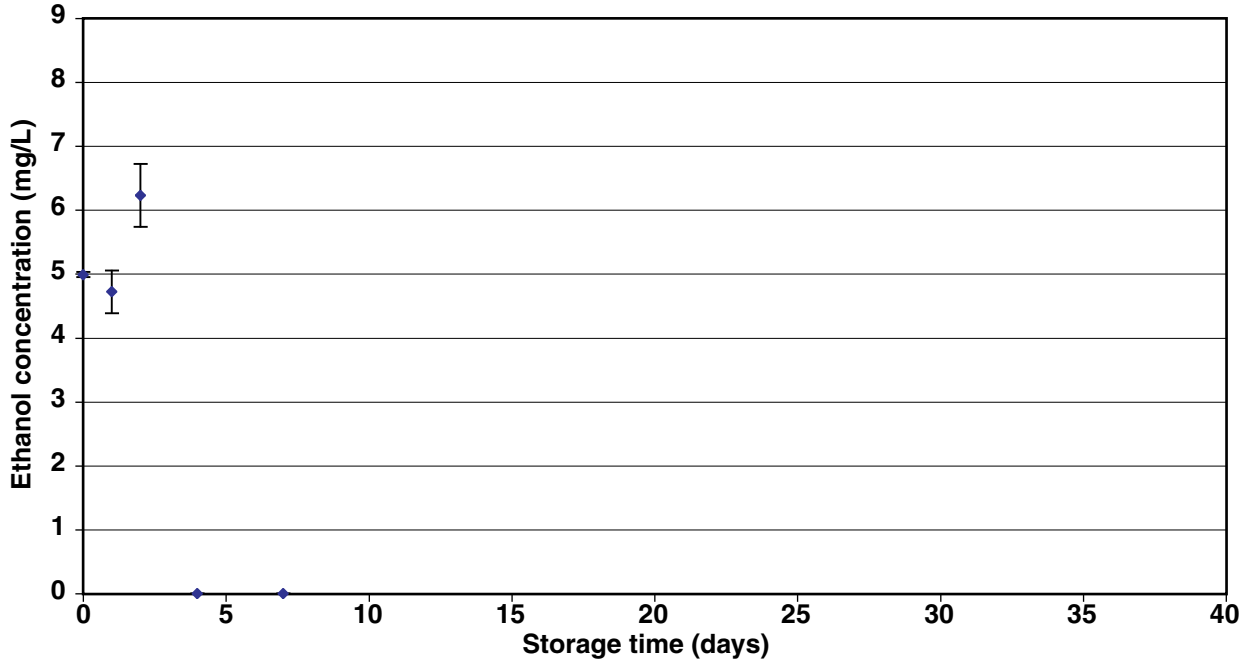

Figure 6-1. Ethanol water sample storage at ambient conditions.

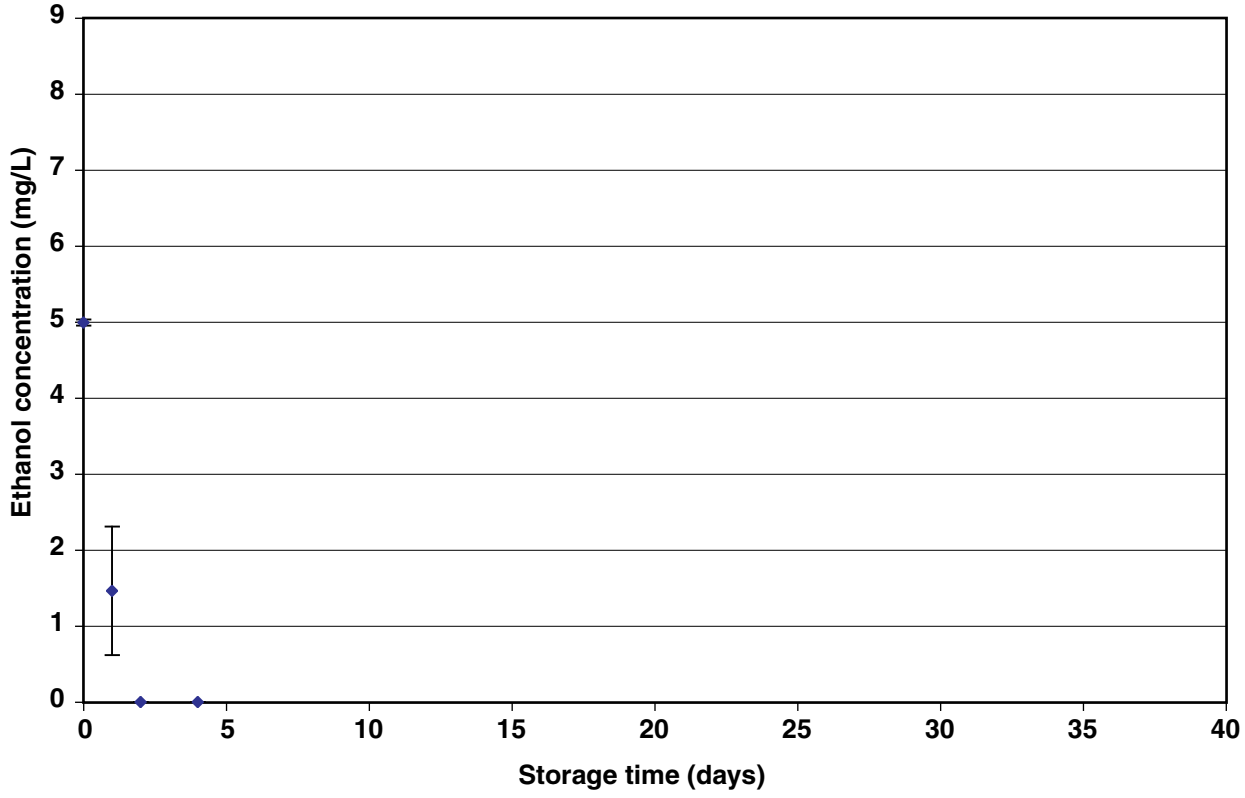

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Figure 6-2. Ethanol water sample storage with refrigeration.

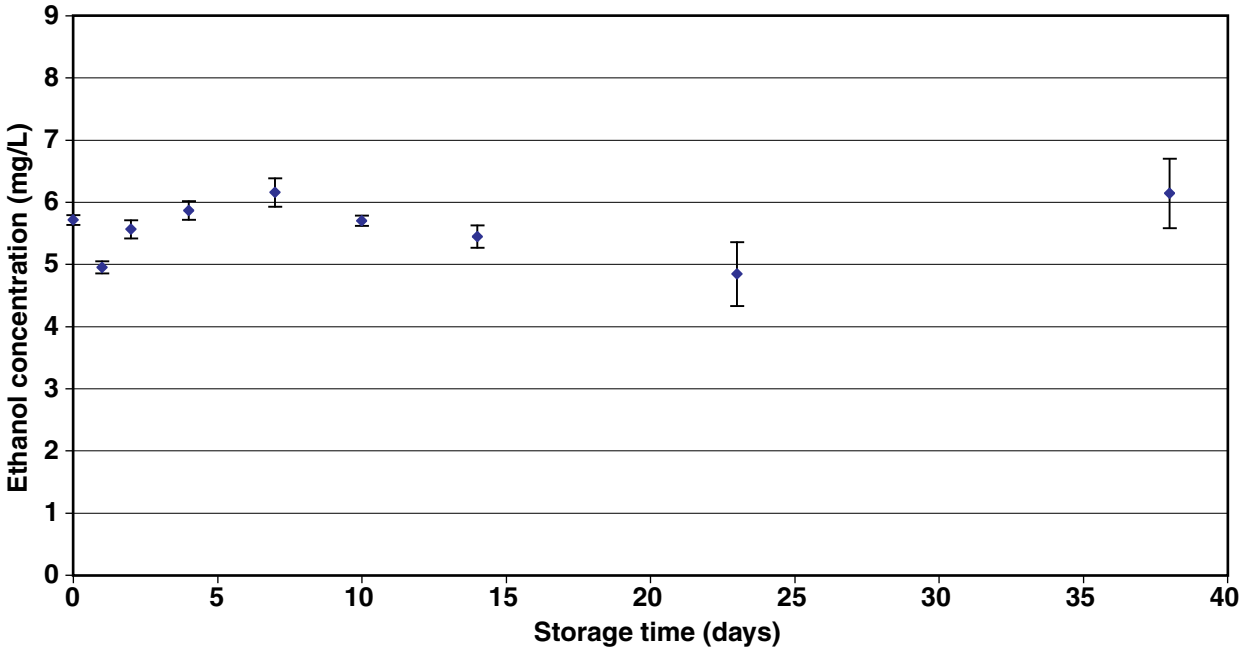

Figure 6-3. Ethanol water sample storage with acid and refrigeration (high concentration).

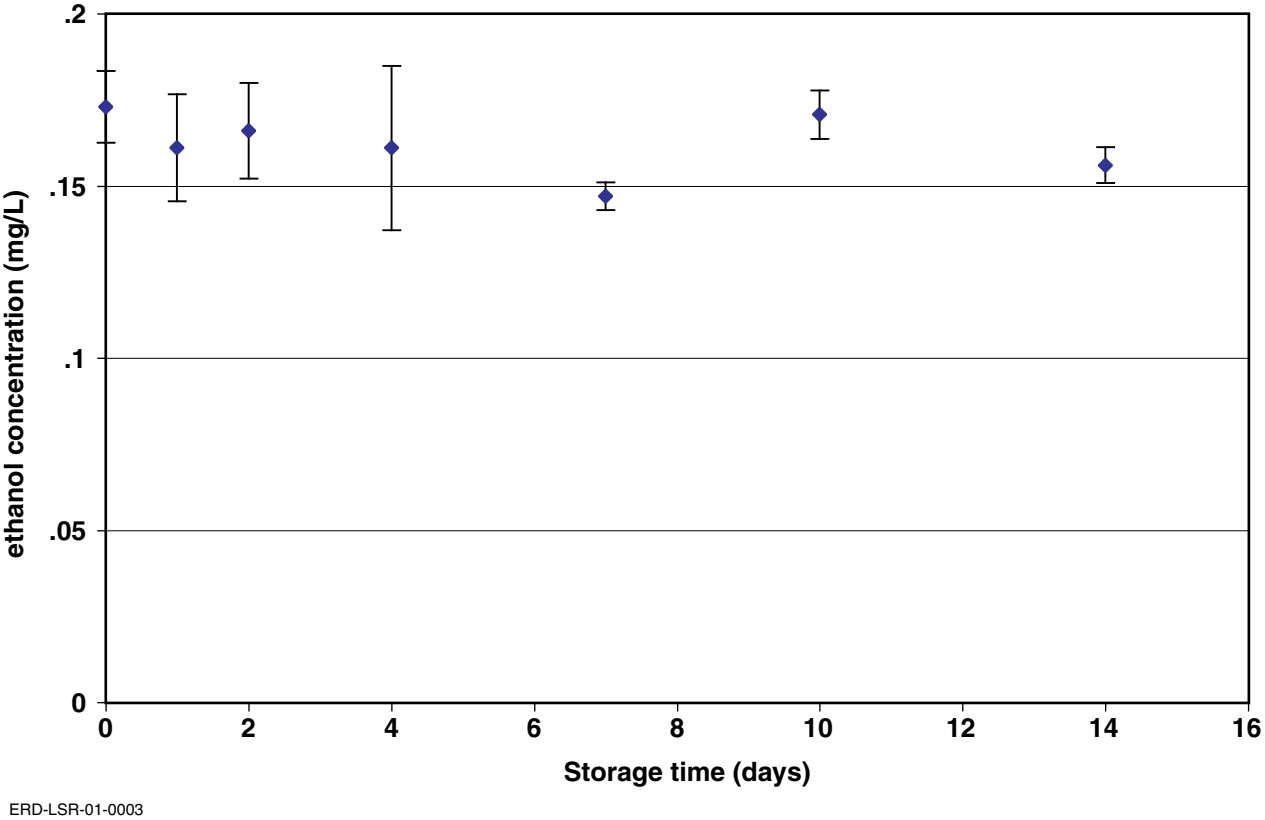

Figure 6-4. Ethanol water sample storage with acidification and refrigeration (low concentration).

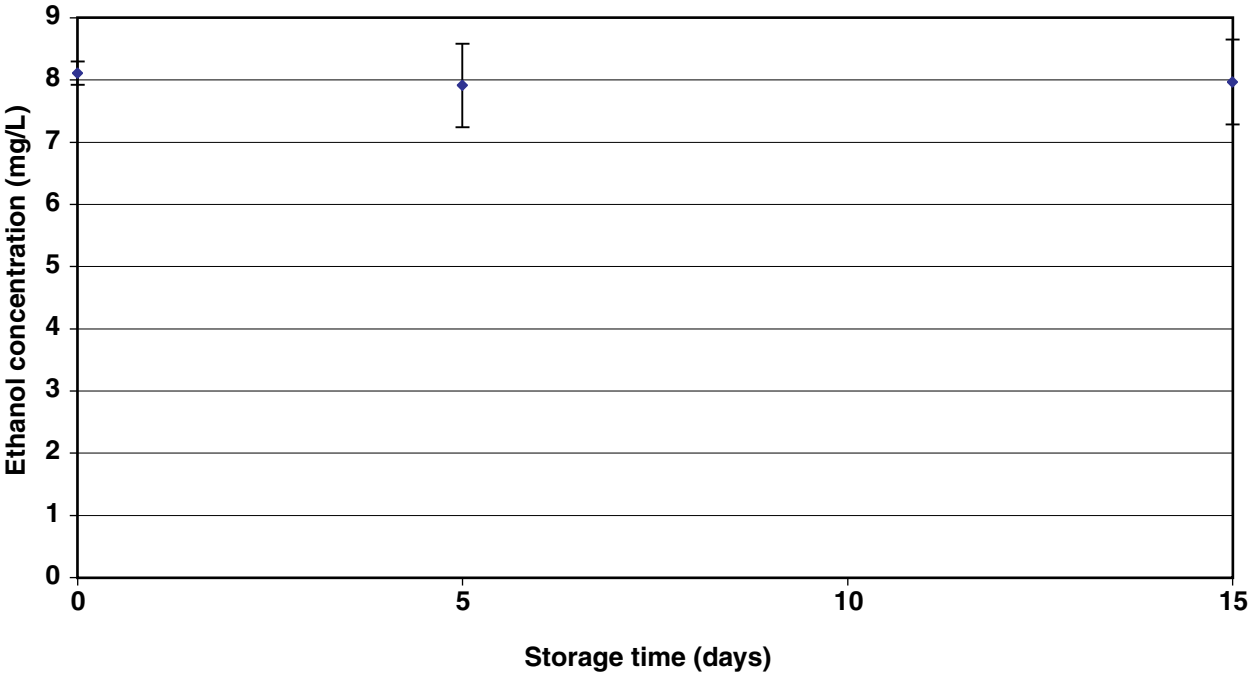

Figure 6-5. Ethanol storage with filtering and refrigeration (high concentration).

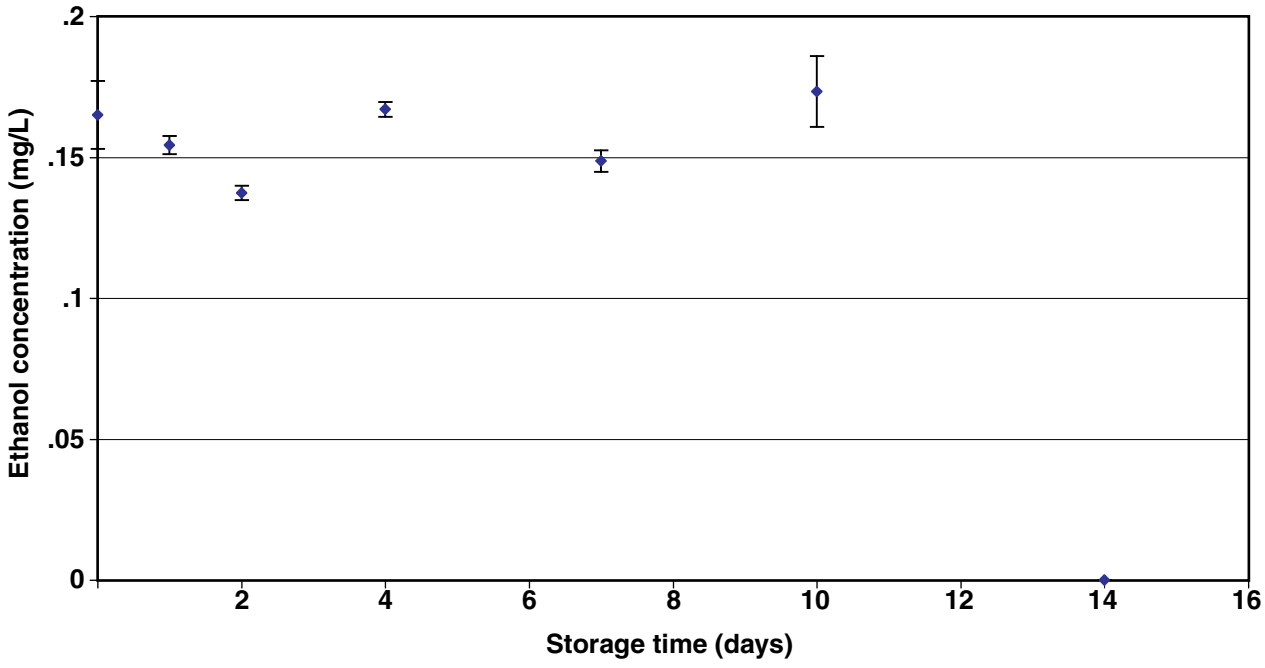

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Figure 6-6. Ethanol storage with filtering and refrigeration (low concentration).

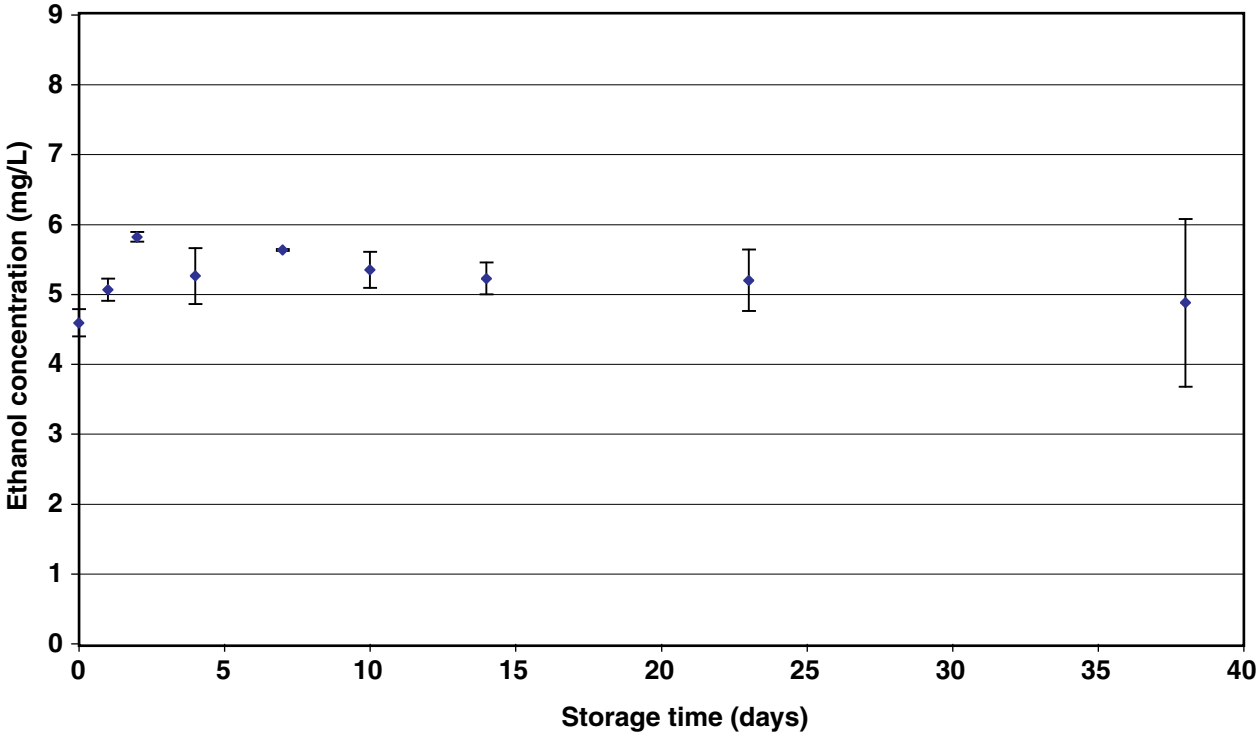

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Figure 6-7. Ethanol water sample storage by freezing.